FIBRIN SELF-ASSEMBLY INHIBITOR TURNOVER IN RATS

E. A. Chiryat'ev, M. K. Umutbaeva, and A. Sh. Byshevskii

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The writers showed previously that human, bovine, and albino rat blood plasma, as well as tissues of the internal organs, contain an inhibitor of fibrin self-assembly, namely a polypeptide with mol. wt. of 1750 daltons (human) and 1278 daltons (rat) [1, 2]. The inhibitor inhibits fibrin self-assembly by forming complexes with intermediate products of that process, not incorporated into the clot [3]. Its concentration changes with age and in certain stress situations and it depends on the animal's position on the "ladder" of evolution [4].

The aim of this investigation was to study changes in concentration of the inhibitor and the mechanism of the changes in diametrically opposite states of blood coagulation, namely when thrombin formation is accelerated and restricted.

EXPERIMENTAL METHOD

A homogeneous preparation of the inhibitor was isolated from plasma obtained from normal blood donors [5]. The concentration of the inhibitor was determined by measuring the degree of inhibition of fibrin self-assembly (from fibrin monomer) and was expressed in conventional units (UC) [1]. Fibrin monomer was obtained from bovine plasma [7]. The inhibitor was labeled with potassium iodide-^{131}I [8] and the radioactivity of the ^{131}I-inhibitor and of material contained it was determined with the DP-100 apparatus, connected to a PP-16 calculator, expressed in cpm/unit of weight or volume.

Turnover of the inhibitor was studied in 640 noninbred albino rats weighing 150-200 g. Excess of thrombin in the blood was created by injecting a commercial preparation of thrombin (activity 7 sec, 1 mg/100 g once daily for 5 days) into the jugular vein. Blood was investigated 1 h after each injection and 1, 2, 3, and 6 days after discontinuing the injections. Restriction of thrombin formation (hypothrombinemia) was induced by Pelentan (5 mg/100 g added to the morning portion of the diet daily from 7 days). Blood samples were taken daily for 12 days. The urine was collected by keeping the animals in metabolism cages and pooling the 24-hourly samples from 10 rats. Before removal of the organs, the animal was perfused through the jugular vein with 0.14 M NaCl solution until the liquid flowing from the right jugular vein was colorless. All the experiments were conducted on animals anesthetized with diethyl ether. The results were subjected to statistical analysis by a method for small series of observations.

EXPERIMENTAL RESULTS

The ^{131}I-inhibitor thus obtained was free from traces of free iodine, as shown by complete agreement between the chromatographic profiles of the specific (antipolymerization) activity and radioactivity of the preparation on gel filtration (Fig. 1).

After intravenous injection of the ^{131}I-inhibitor (0.5 ml, 138,000 cpm/100 g) the radioactivity of the blood 30 sec later was 21,540 cpm/ml, and the corresponding values 1.5, 3, 5, 11, 20, and 60 min later were 52.8, 16.2, 15.8, 14.8, 8.4, and 6.5%, respectively, of this value, which reflected the initial blood level of the inhibitor. Radioactivity of the organs (liver, lungs, heart, brain, kidneys, spleen, small intestine, striated muscle, and subcutaneous cellular tissue) 1 h after the injection varied from 75 to 550 cpm/g wet weight of tissue. Consequently, the inhibitor was very quickly, but not equally quickly, removed from the blood by different tissues.

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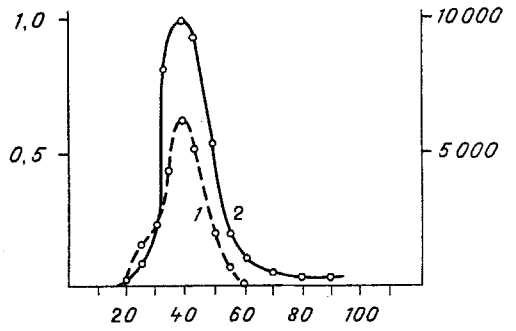

Fig. 1

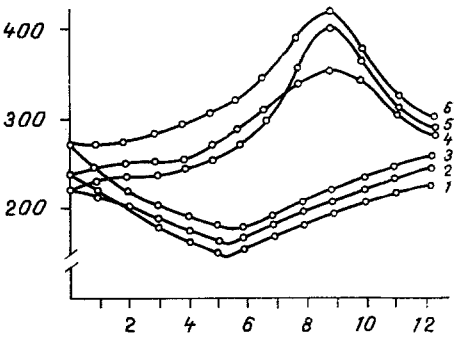

Fig. 2

Fig. 1. Chromatographic profiles of antipolymerization activity (1) and radioactivity (2) during gel filtration of preparation on Sephadex G-15. Abscissa, volume of eluent (in ml); ordinate: on left — efficiency of inhibition (in CU), on right — radioactivity (cpm/ml).

Fig. 2. Change in inhibitor concentration in lungs, liver, and heart against the background of thrombinemia (1, 2, and 3, respectively) and after injection of Pelentan (4, 5, and 6). Abscissa, time after stopping injections of thrombin (in days); ordinate, inhibitor concentration (in Cu).

When the inhibitor was injected subcutaneously (posterior surface of the thigh, 0.5 ml, 111,200 cpm) the radioactivity of the blood 6 min later was 665 cpm/ml and it did not change significantly in the next 2 h; after 20, 40, 60, 90, and 120 min it was 785, 720, 730, 700, and 750 cpm/ml, respectively. The radioactivity of the tissues after 2 h was as follows (in cpm/g wet weight of tissue): lungs 400 ± 32, heart 176 ± 19, liver 250 ± 21, small intestine 396 ± 30, striated muscle 290 ± 19, brain 176 ± 11, kidneys 545 ± 46, spleen 425 ± 41, subcutaneous cellular tissue 370 ± 23. It can be concluded that the inhibitor passes continuously from the subcutaneous cellular tissue into the blood stream, and equally quickly from the blood stream into the organs studied. This determined the constant concentration of the inhibitor in the blood steram when a local depot existed in the subcutaneous cellular tissue.

Injection of thrombin into the rats in these experiments caused no appreciable changes in the blood level of the inhibitor. The level of the inhibitor in the tissues fell appreciably and remained low for 4-5 days after withdrawal of the thrombin (Fig. 2: 1-3). After injection of Pelentan (hypothrombinemia) the plasma level of the inhibitor likewise was unchanged, but the level in the tissues rose, and did not return to normal until a few days after discontinuation of the Pelentan (Fig. 2: 4-6).

The formation of fibrinogen metabolites is accelerated in hyperthrombinemia. Accordingly the plasma level of the inhibitor ought evidently to fall as a result of its loss by association with fibrinogen conversion products [3], which are removed from the blood by passage into the lymph or are ingested by cells of the reticuloendothelial system [6]. In fact the level of the inhibitor remained constant in the plasma but fell in the tissues. This suggested that the tissues compensate for continuous loss of plasma inhibitors. This explanation is in agreement with the changes discovered in hypothrombinemia (due to Pelentan): under those conditions losses of the inhibitor due to the formation of association with fibrinogen conversion products were reduced, the rate of arrival of inhibitor from the tissues fell correspondingly, and, consequently, accumulation of the inhibitor could take place in the tissues.

The following experiments demonstrate the possibility of rapid recovery of the inhibitor concentration in the tissues: 5 min after replacement of 40% of the circulating blood with 0.14 M NaCl solutions the inhibitor concentration in the plasma fell from 39 ± 0.2 to 31 ± 0.1 CU/ml, after further replacement of the blood by another 25% the inhibitor concentration fell to 29.0 ± 0.3 CU/ml, but another 30 min later it had recovered to 35.0 ± 0.2 CU/ml, and 60 min later to 40.0 ± 0.2 CU/ml. These data and those given above show that the inhibitor can be rapidly distributed between the tissues of organs and the blood in both directions.

After injection of thrombin and also of Pelentan excretion of the inhibitor with the urine increased after 3-8 and 4-11 days, respectively (Table 1).

TABLE 1. Content of Inhibitor (in CU) in 24-Hourly Urine Sample of Albino Rats, Calculated per Animal Weighing 100 g, after Injection of Thrombin or Pelentan (M ± m, n = 9-11)

Time after beginning of exp. days	Control	Injection of thrombin	Injection of pelentan
1	171±3	178±5	177±4
2	155±4	170±4	184±2
3	148±6	198±5*	209±3
4	188±4	238±3*	275±6*
5	218±5	288±5*	280±5*
6	208±6	305±8*	302±3*
7	169±7	196±7*	405±4*
8	166±2	229±4*	506±4*
9	177±5	217±5	574±1*
10	176±5	198±5	477±5*
11	181±6	—	329±5*
12	169±5	—	192±6

Legend. *p < 0.05.

The more rapid excretion of the inhibitor with the urine raises doubts about the view that it is eliminated more rapidly with products of fibrin self-assembly. May not the content of the inhibitor in the tissues be reduced on account of more rapid excretion with the urine? The following calculation provides the answer to this question. In the course of 4, 5, and 6 days of the experiment after injection of thrombin the rats excreted with the urine 327 CU of inhibitor more than in the control. The inhibitor content in the liver during this period fell on average by 209 CU (72 CU/g tissue). If the loss of inhibitor in the tissues of other organs is added to this value, it will be clear that the loss of inhibitor by the tissues is greater than its amount excreted with the urine. The view that the inhibitor is removed more rapidly during hyperthrombinemia with products of fibrin self-assembly has some basis.

It seems paradoxical that in hypothrombinema (after injection of Pelentan), when the inhibitor is excreted more rapidly with the urine, its content in the tissues rises. However, whereas under normal conditions the inhibitor is removed in the form of associations with continuously formed products of fibronogen conversion when coagulation is blocked this pathway is closed or delayed, of excretion of the inhibitor with the urine is accelerated. The degree of acceleration of excretion ofthe inhibitor with the urine is evidently small compared with its loss by coagulation. Due to blockage of the latter pathway, passage of the inhibitor from the tissue into the blood is delayed, and as a result, it accumulates in the tissues.

On the basis of data showing an increase in the content of the inhibitor after the end of thrombin injection enable the rate of respiration of the tissue reserves of the inhibitor to be assessed. In the 6 days after discontinuation of thrombin injections the content of inhibitor in the brain increased by 22%, in the aorta by 38%, in the intestinal wall by 23%, in the spleen and striated muscle by 39%, in the liver by 27%, and in the myocardium by 32%. Because the losses are restored so quickly, the reduction of the reserves of inhibitor during hyperthrombinemia can be compensated and their accumulation during hypothrombinemia ensured.

Besides information on the relationship between age, the state of blood coagulation, and the level of evolutionary development of animals, on the one hand, and the inhibitor content on the other hand [4], the results described above indicate a role of the inhibitor in the regulation of clotting activity of the blood at the level of its final, phylogenetically oldest stage. Maintenance of a constant inhibitor level in the blood despite considerable shifts during blood clotting and rapid recovery, even after replacement of more than 50% of the blood volume with isotonic solution, are evidence of the important role of this peptide anticoagulant.

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EFFECT OF PLASMA FLOW ON REGENERATION OF SKIN WOUNDS AND ON REACTIVITY OF THE BODY

I. V. Stupin. N. P. Mikaelyan, M. I. Ul'yanov, and G. G. Belous

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The introduction of the "plasma scalpel," whose action is based on the use of the flow of energy of ionized gases, into surgical practice for dividing biological tissues and treating wounds [1], necessitates a deeper study of this subject.

The aim of this investigation was to study the character of action of the plasma flow on biological structures, on individual systems, and on the body as a whole.

EXPERIMENTAL METHOD

The effect of the flow of a plasma jet on wound healing was studied in three series of experiments (six rabbits in each series). In series I and II, under aseptic conditions, wounds were inflicted on the animals' back by removal of flaps of skin and fascia measuring 4 × 4 cm. In series III, only the hair was removed from the animals in the corresponding region. Daily, starting from the 1st day, the wound region in the animals of series II and the intact skin of rabbits in series III of the experiments were "irradiated" with a plasma flow. The temperature of the plasma flow at the site of contact with the animal's tissues was 37-38°C. The wounds healed by the open method. Assessment of the character of wound healing was based on times of regeneration, planimetry of the wound, and cytology of the wound exudate. Reactivity of the body was judged on the basis of the study of peripheral blood and bone marrow morphology, determination of proteins, glucose, and lipids in the blood plasma, and investigation of lipid peroxidation (LPO) by determining accumulation of malonic dialdehyde (MDA) in the blood.

EXPERIMENTAL RESULTS

Immediately after wounding of the rabbit a vasomotor response developed at the site of the defect and exudation of the wound took place. After the end of 3 days, in the experiments of series I a marked inflammatory reaction developed, accompanied by hyperemia of the edges and floor of the wound defect, with features of hydration. From the 3rd through the 7th day after wounding, healing took place in all the animals in this series of experiments, beneath a scab. Later, the scab on one animal was rejected and the wound granulated openly until epithelization was complete. Granulation processes and epithelization took place in the other animals beneath the scab.

In the experiments of series II, on the 2nd day after application of the plasma flow, features of exudation in the wound region were mild in degree, and later a delicate, thin scab formed. The wounds contracted well and healed with an accurate scar, almost indistinguishable in appearance from the surrounding skin. The area of the wound in this series of experiments decreased by 47% until the 9th day, whereas in the animals of series I it decreased by only 22%. The mean rate of regeneration in series I, without treatment by plasma flow, was 2.9% in 24 h, compared with 6.1% after treatment with plasma.

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